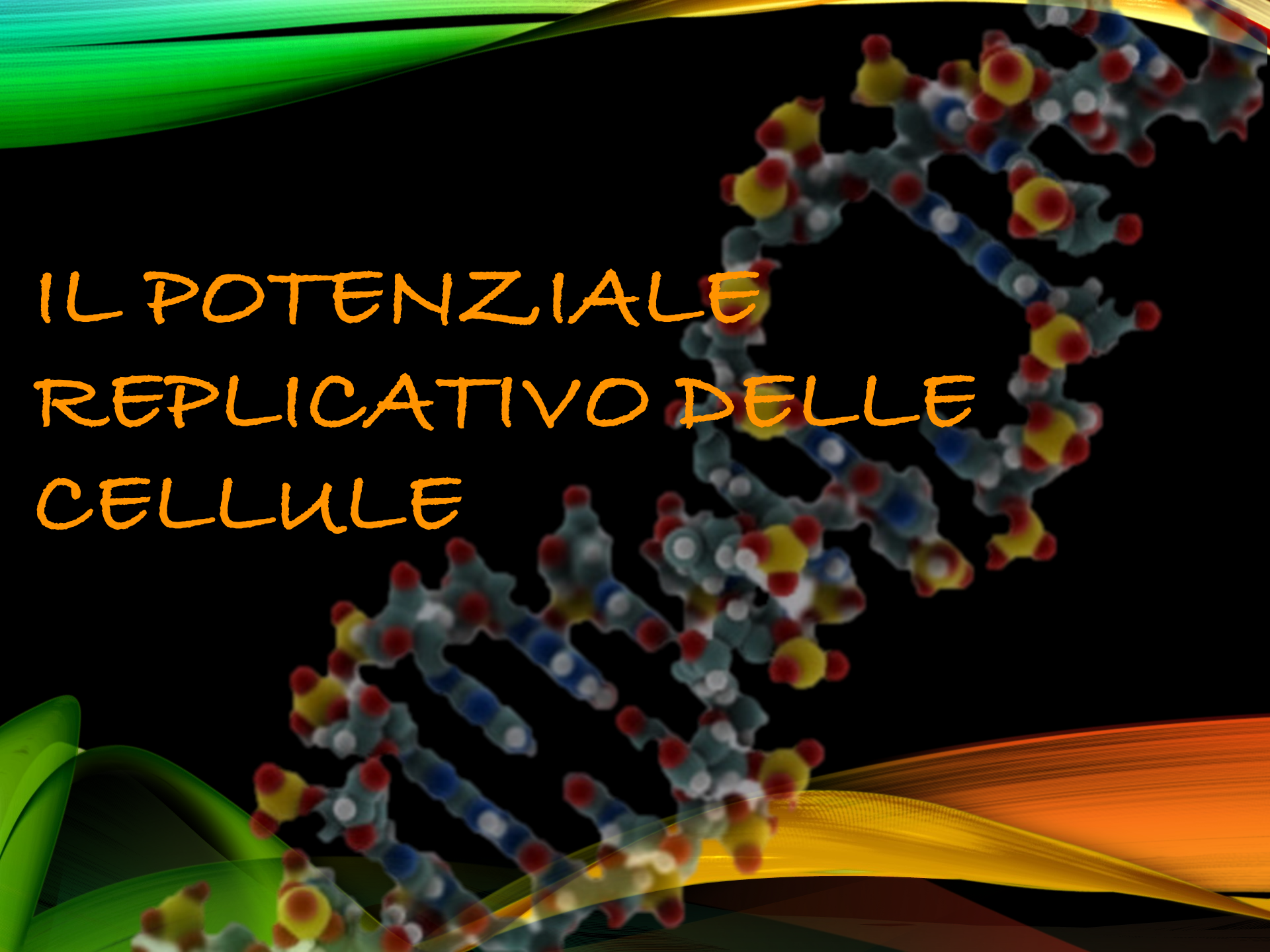
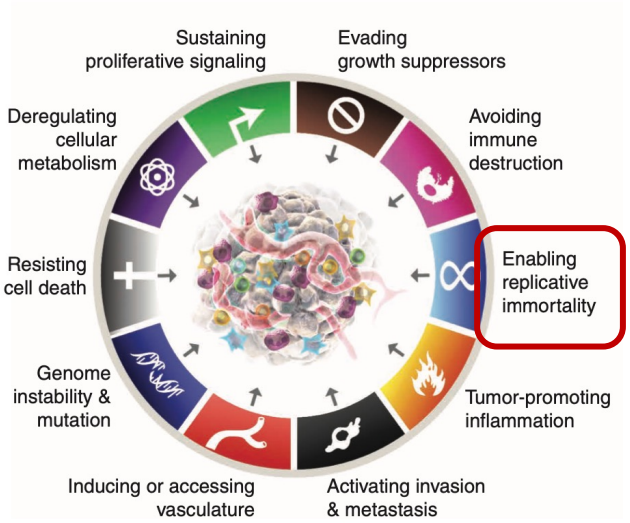
I TELOMERI E L'IMMORTALIZZAZIONE DELLE CELLULE TUMORALI

IL POTENZIALE REPLICATIVO DELLE CELLULE



Potenziale replicativo



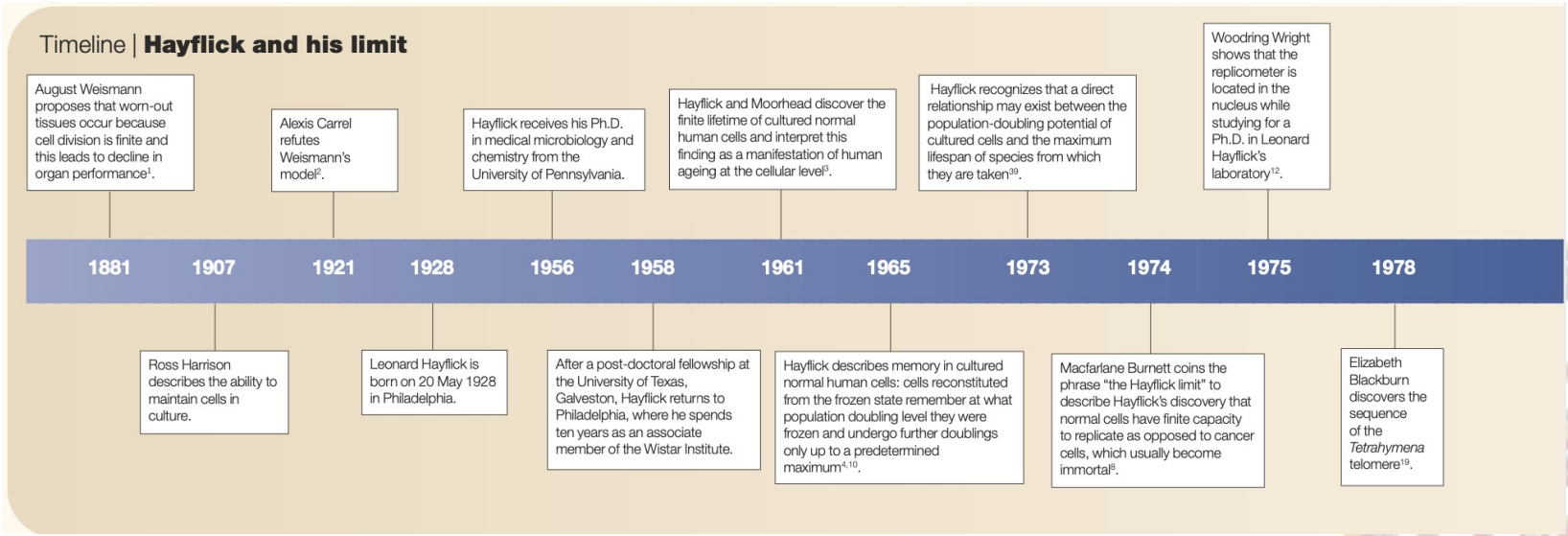
Cellule normali non possono dividersi all'infinito

Cellule tumorali → molti cicli di divisone

TIMELINE

Hayflick, his limit, and cellular ageing

Jerry W. Shay and Woodring E. Wright



Shay & Wright, 2000

The Hayflick's limit

Le cellule possono duplicarsi all'infinito se non hanno limiti imposti dall'esterno?

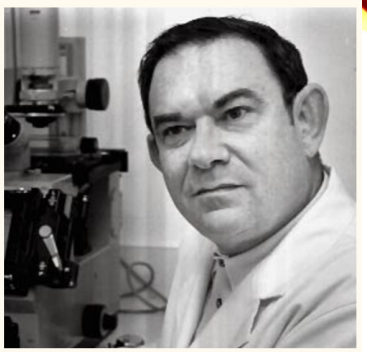


Figure 1 | **Leonard Hayflick in 1988.**
(Photograph: Peter Argentine.)

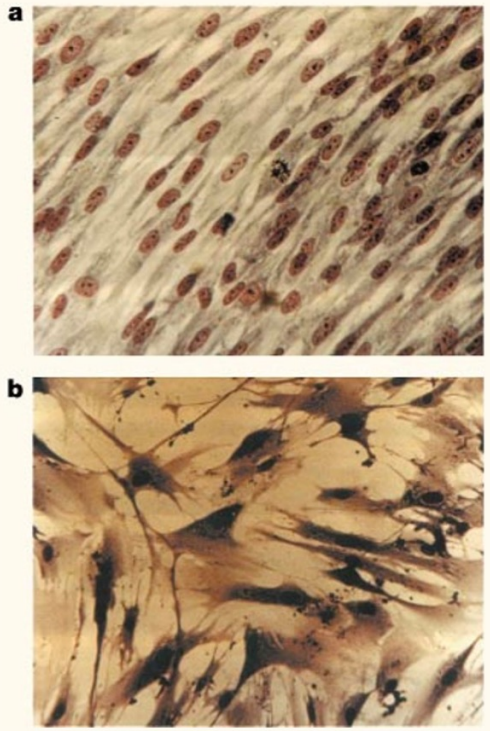


Figure 2 | **Young and old human diploid cells (strain WI-38). a** | Young cells in phase II at population doubling 20. **b** | Old cells in phase III at population doubling 55.

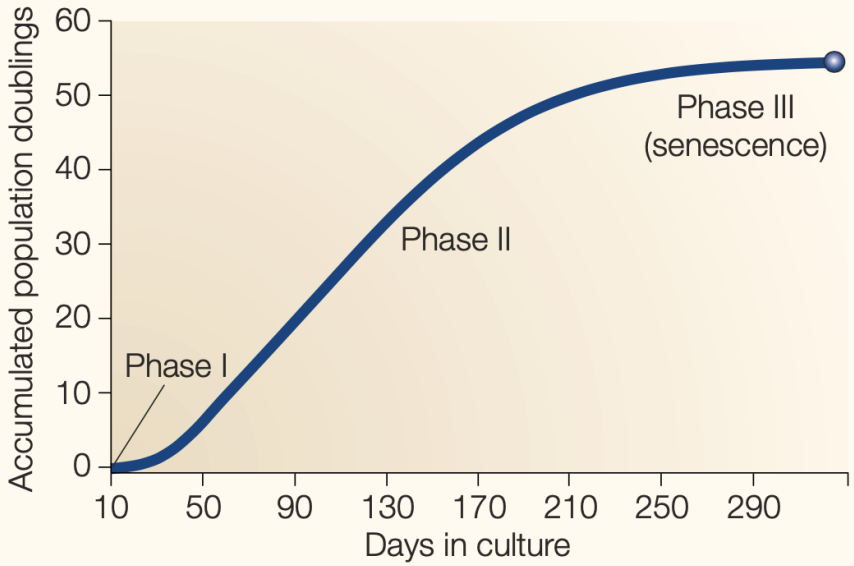


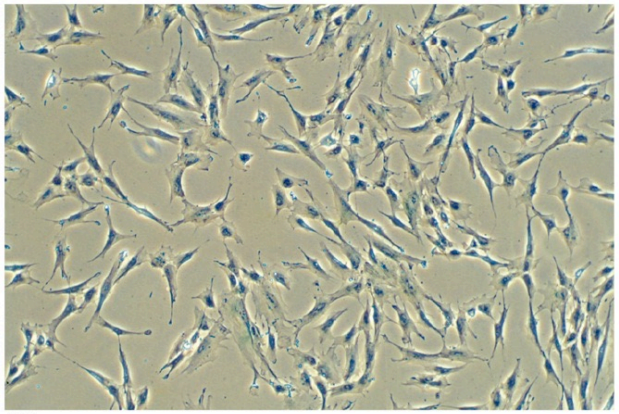
Figure 3 | **Hayflick's three phases of cell culture.** Phase I is the primary culture; phase II represents subcultivated cells during the period of exponential replication. Phase III represents the period when cell replication ceases but metabolism continues. Cells may remain in this state for at least one year before death occurs.

Shay & Wright, 2000

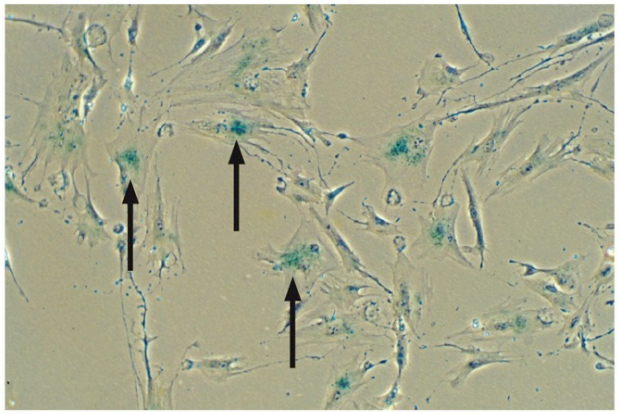
The Hayflick's limit

Le cellule invecchiano! Cellule normali fanno fino a 50-60 cicli di divisione prima di entrare in senescenza

Cellule vecchie esprimono β-galattosidasi



(A)



(B)

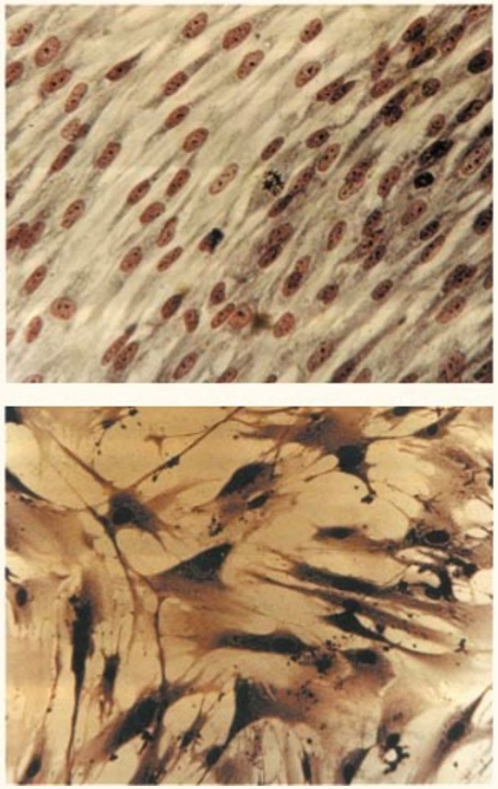


Figure 2 | **Young and old human diploid cells (strain WI-38). a** | Young cells in phase II at population doubling 20. **b** | Old cells in phase III at population doubling 55.

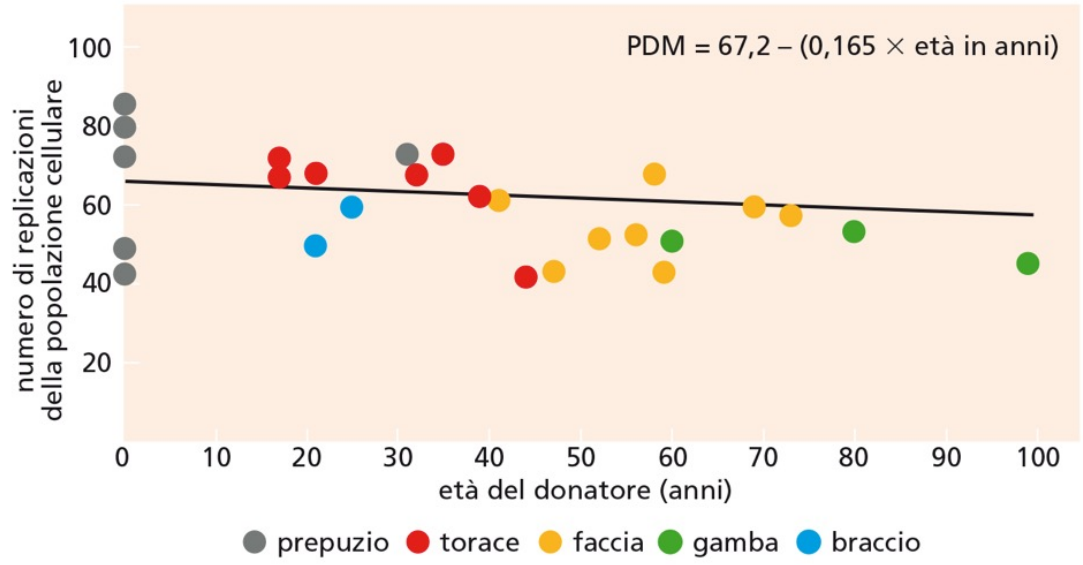
N.B. Cellule ES sfuggono a questa regola

Weinberg, la biologia del cancro; Shay & Wright, 2000

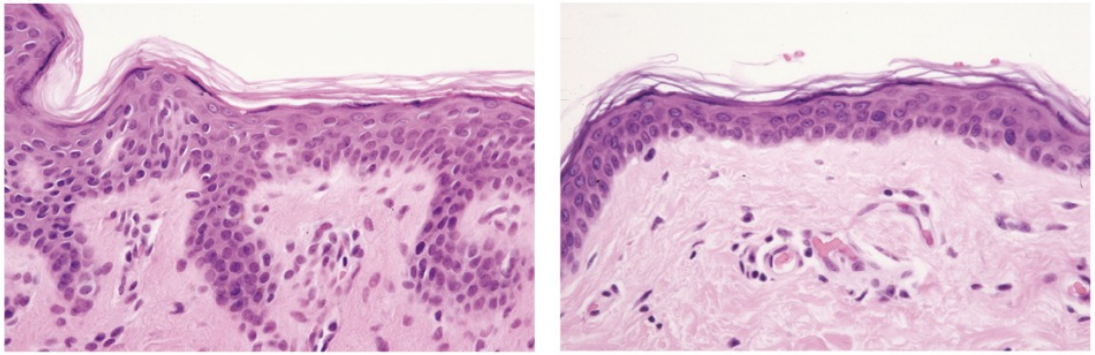
The Hayflick's limit

Perdita della capacità proliferativa con avanzare dell'età

(A)

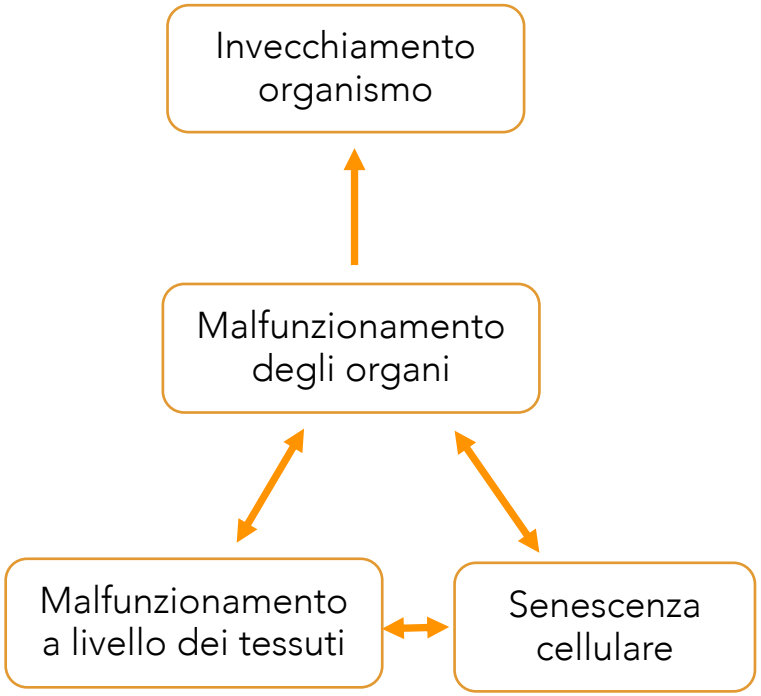


(B)



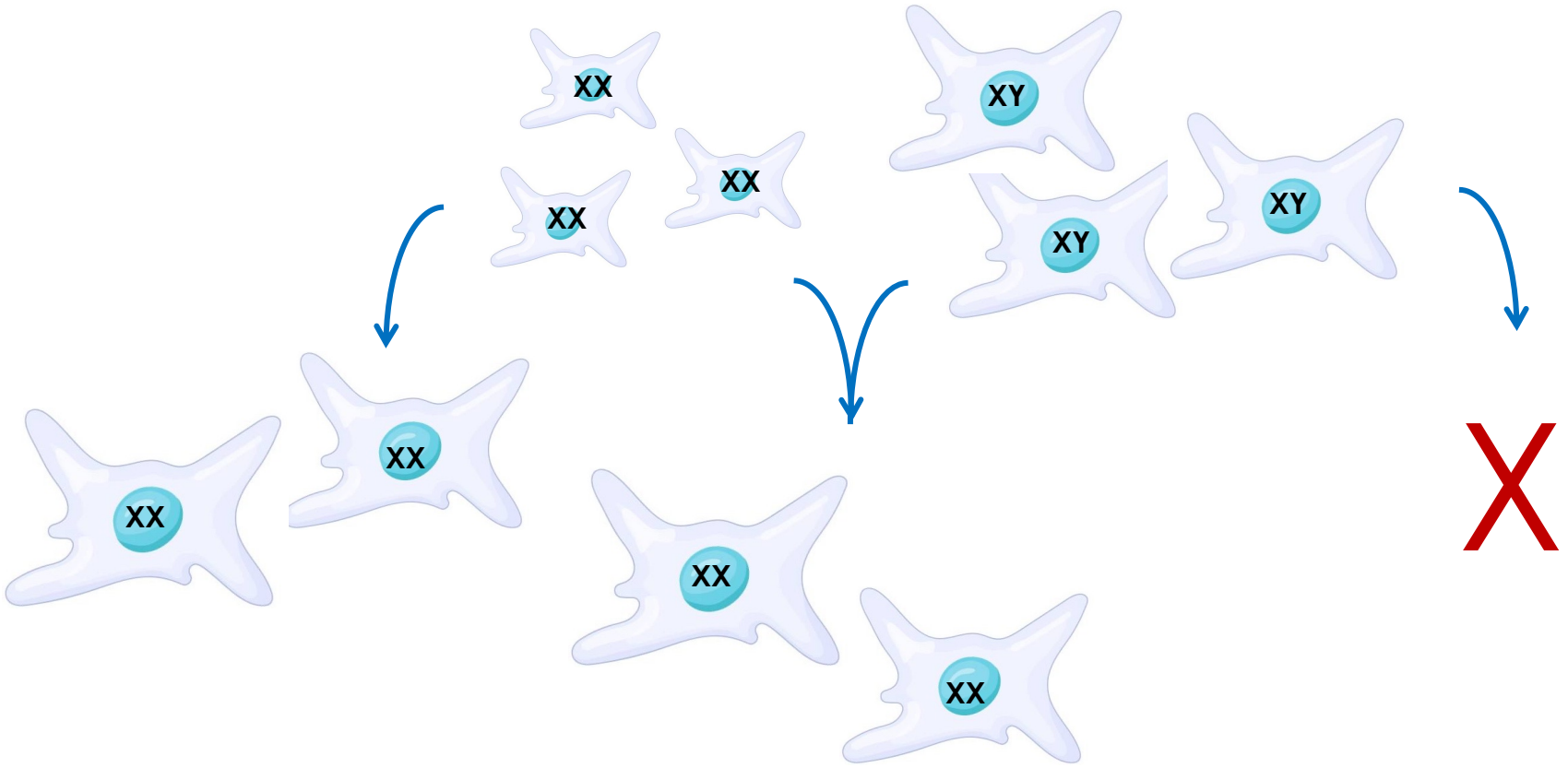
18 anni

76 anni



Weinberg, la biologia del cancro

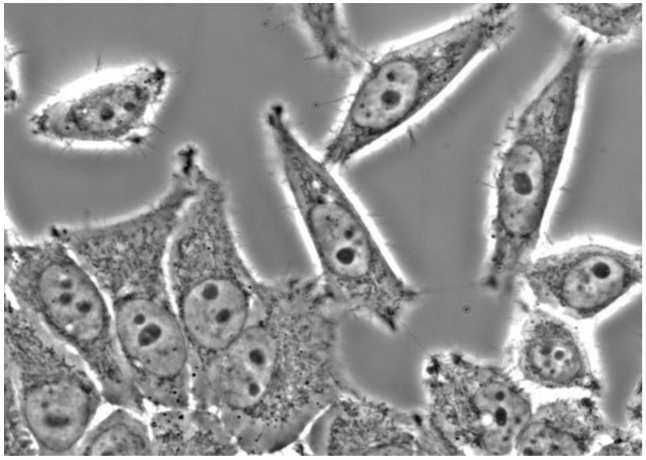
Memoria proliferativa



The Hayflick's limit

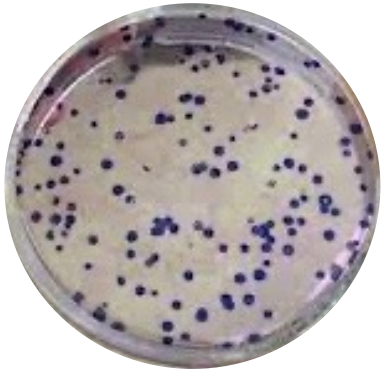
Cellule ES e cellule tumorali sfuggono a questa regola

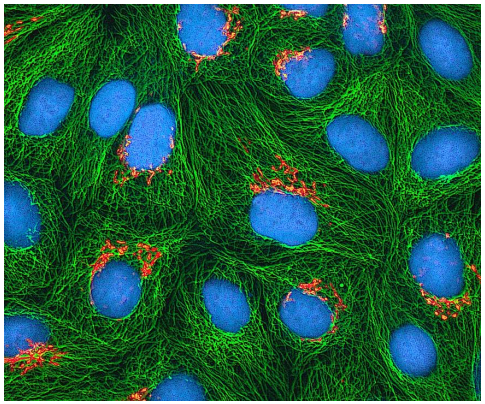
HeLa cells



HeLa cells in cell culture; photo taken with a phase contrast microscope

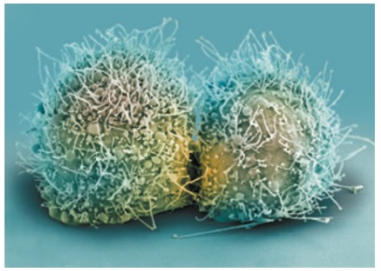
Henrietta Lacks with her husband David.





THE LACKS LEGACY

Story of the world's most widely used human biological research tissue.



1951 Biopsy of Henrietta Lacks' tumour collected without her knowledge or consent. HeLa cell line soon established.

1971 The journal *Obstetrics and Gynecology* names Henrietta Lacks as HeLa source; word later spreads in *Nature, Science* and mainstream press.

1973 Lacks family members learn about HeLa cells (pictured). Scientists later collect their blood to map HeLa genes, without proper informed consent.

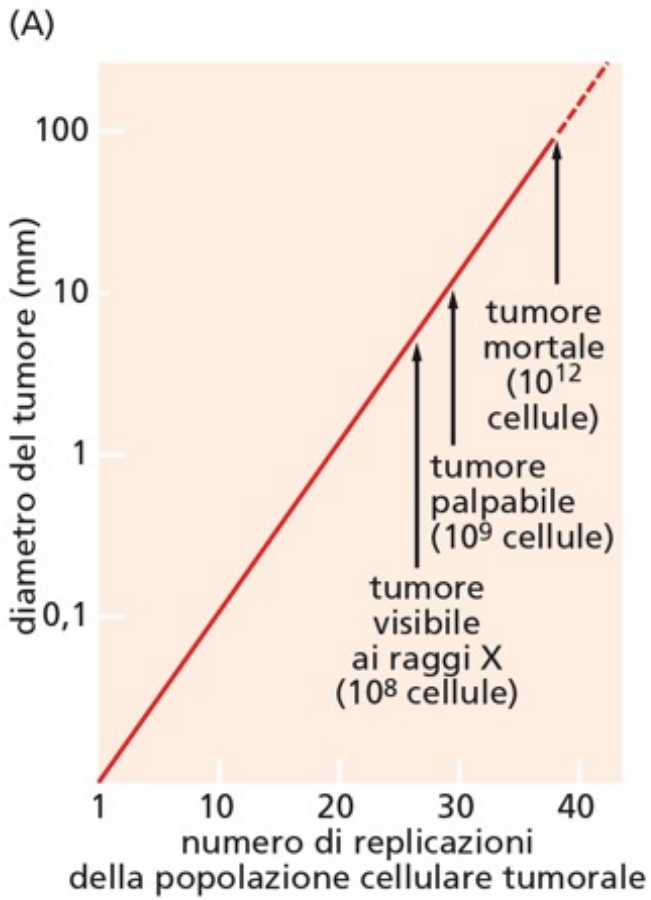
1996 Lacks family honoured at the first annual HeLa Cancer Control Symposium, organized by former student of scientist who isolated HeLa cells.

2013 HeLa genome published without knowledge of the family, which later endorses restricted access to HeLa genome data.

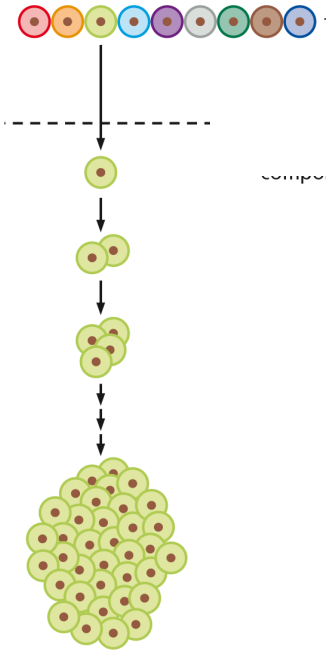
https://www.gesundheitsindustrie-bw.de/en/article/news/hela-a-human- bauplan-in-the-petri-dish

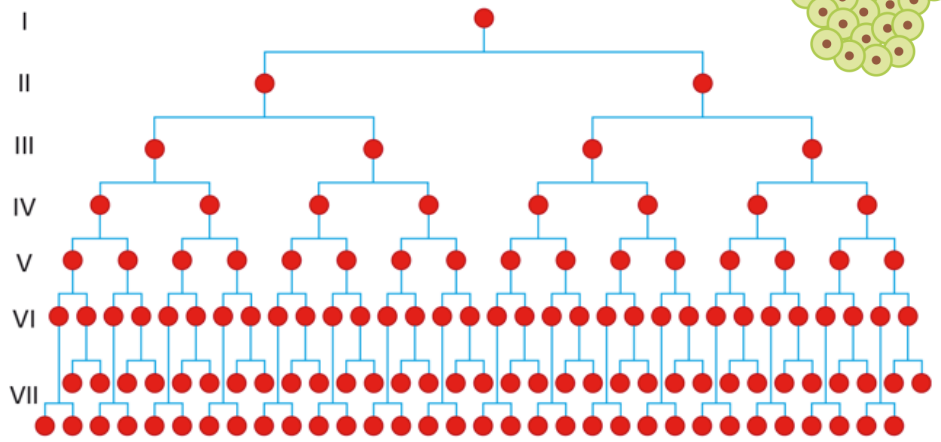
Quante generazioni servono per fare un tumore?

Tumore è clonale: cellule della massa tumorale derivano da una cellula ancestrale



10^{12} => 2^{40} cellule
Formazione tumore mortale richiede 40 generazioni?

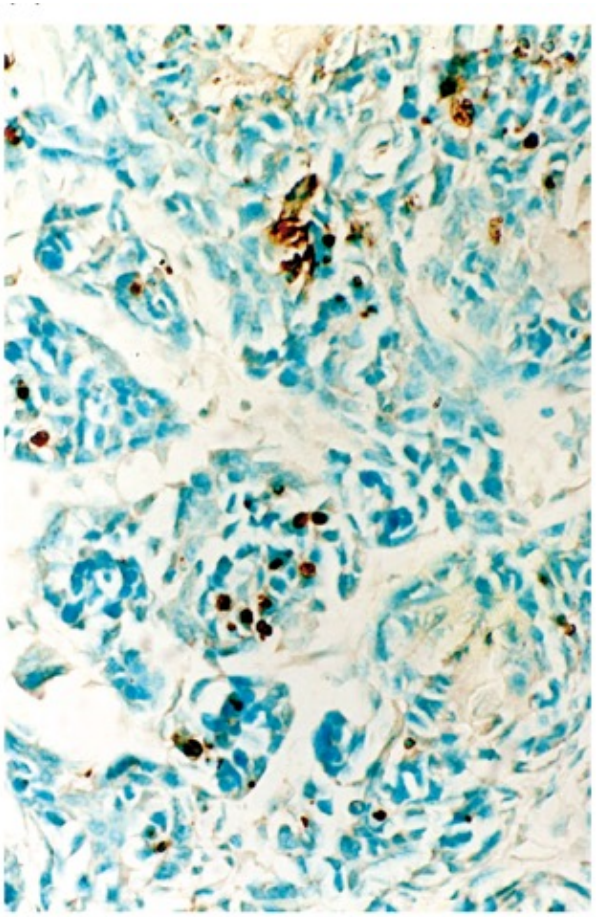


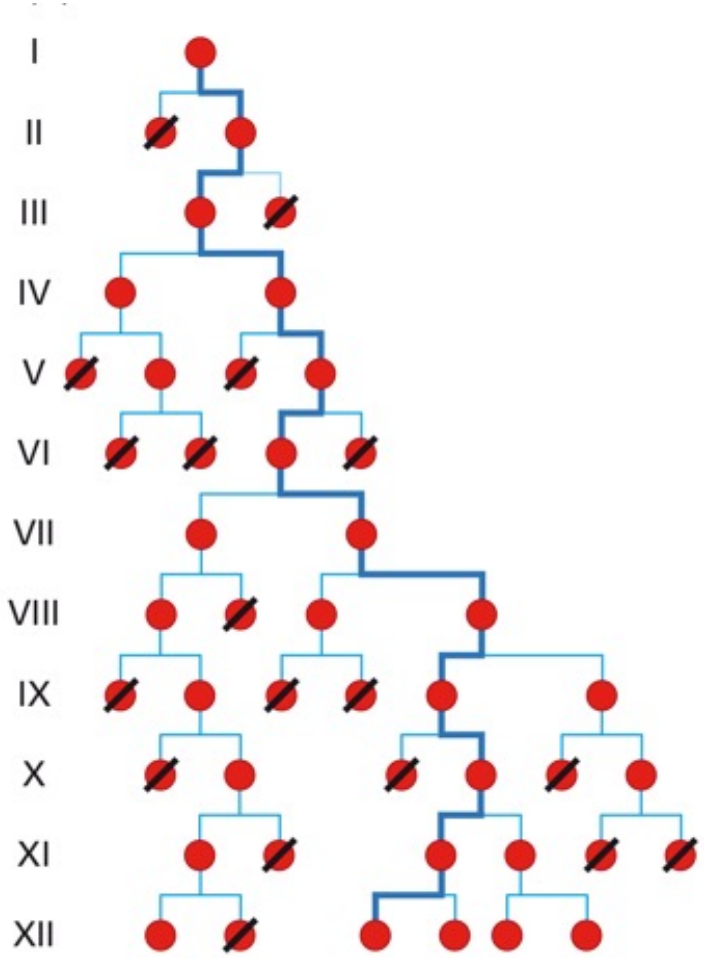


Weinberg, la biologia del cancro

Quante generazioni servono per fare un tumore?

In realtà molte di più: la crescita delle cellule tumorali non è esponenziale, almeno all'inizio



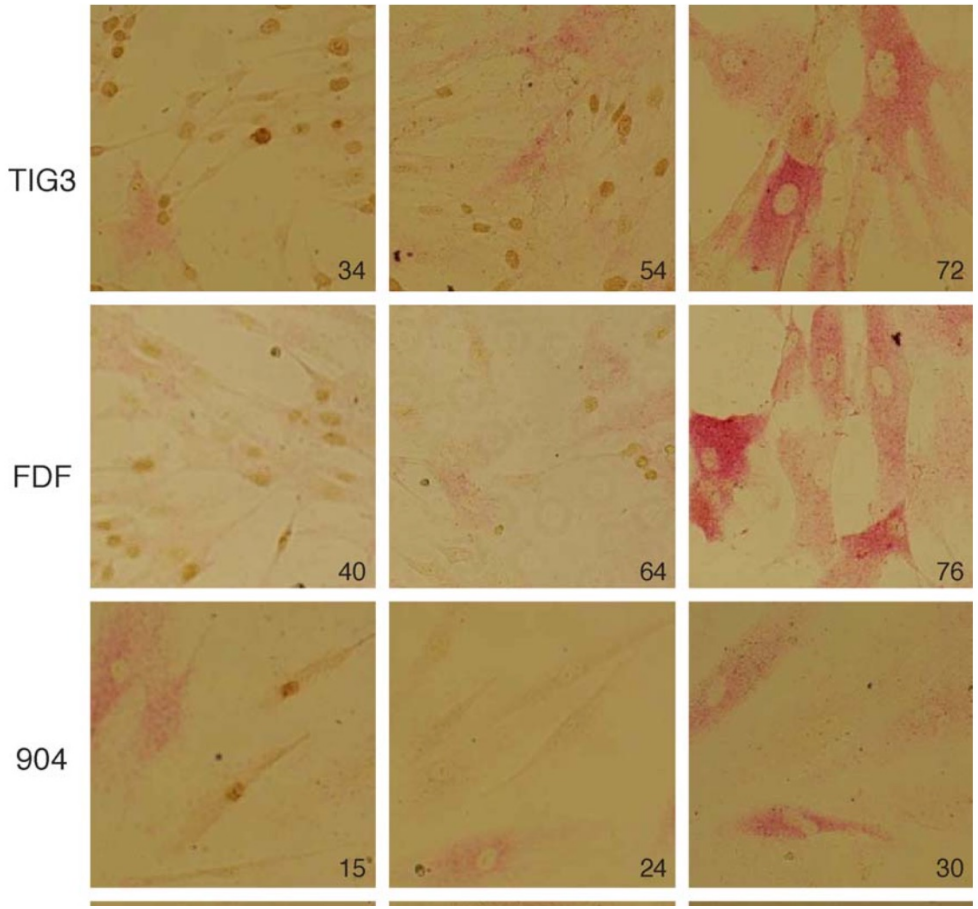


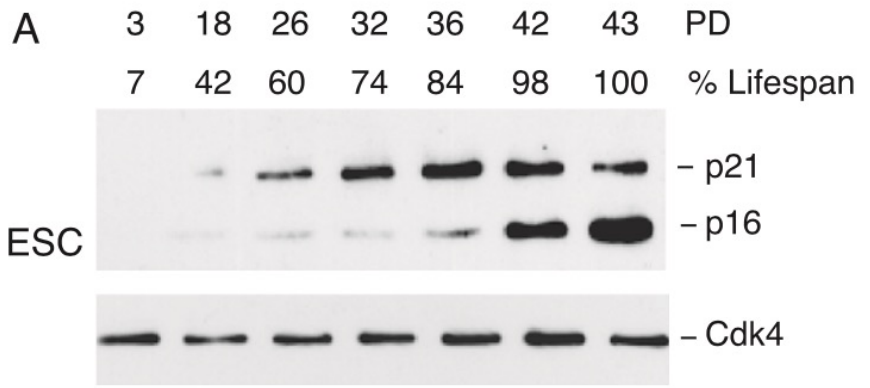
Weinberg, la biologia del cancro

COME LE CELLULE CONTANO LE GENERAZIONI E ATTIVANO LA SENESCENZA?

Elevati livelli di CDKI in senescenza

Immunohistochemical staining for $p16^{INK4a}$ using the JC8 antibody and alkaline phosphatase secondary detection system (red) and for Ki67 using HRP-based detection (brown).





Brookes et al., 2004; Chen et al., 2013

Elevati livelli di CDKI in senescenza

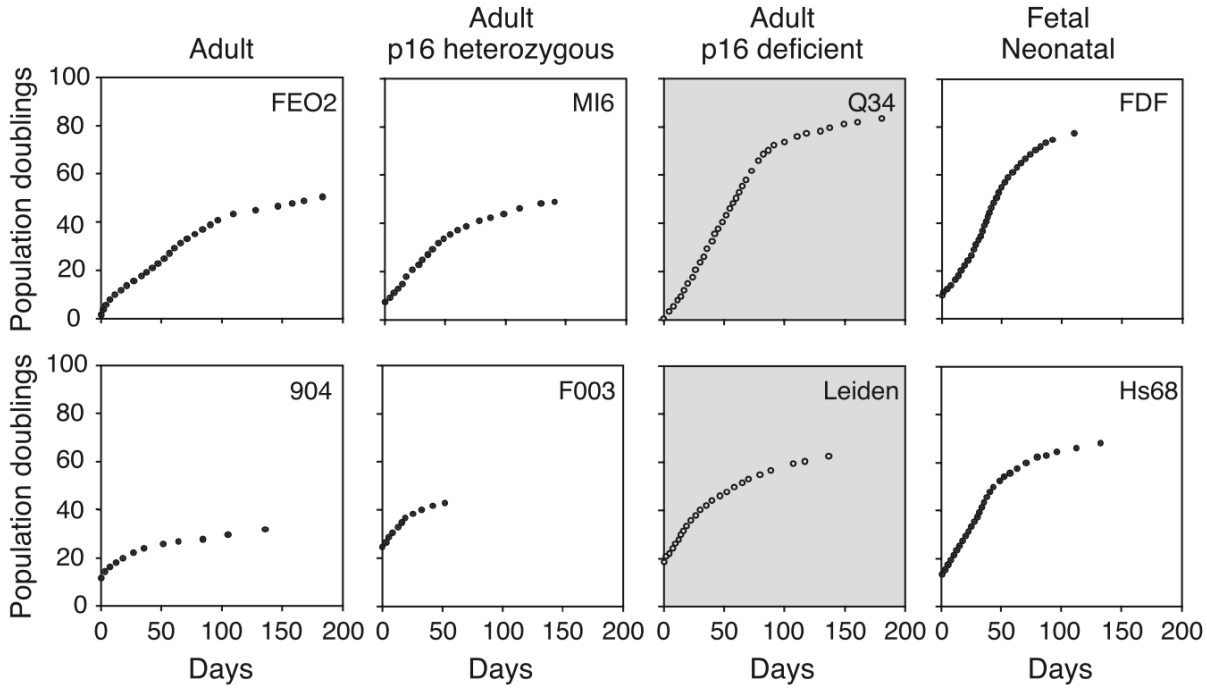


Fig. 1. Limited life spans of different strains of dermal fibroblasts. Each panel shows the cumulative populations doublings (PDs) achieved by the indicated strain of human fibroblast as a function of time. The two $p16^{INK4a}$-deficient strains are identified by the grey background. Note that depending on the source of the cells, the zero time points reflect different numbers of PDs. The results are representative of several independent growth curves as summarized in Table 1.

$$D = C + n + \left[\frac{B - A(2^n)}{A(2^{n+1}) - A(2^n)} \right]$$

Figure 3.
Equation to determine population doubling of aging cells in culture. Where A equals no. of cells plated, B equals no. of cells counted after growth period, C equals old population doubling, D equals new population doubling and *n* equals the largest number that satisfies the equation $A(2^n) \leq B$.

Brookes et al., 2004; Chen et al., 2013

Indicatori di senescenza

H_2O_2-induced senescence

Biochemical characteristics of cellular senescence:
-elevated protein levels of p53, $p16^{INK4a}$, promyelocytic leukemia gene product PML
-senescence-associated h-galactosidase (SA-h-gal) activity assayed at an acidic pH

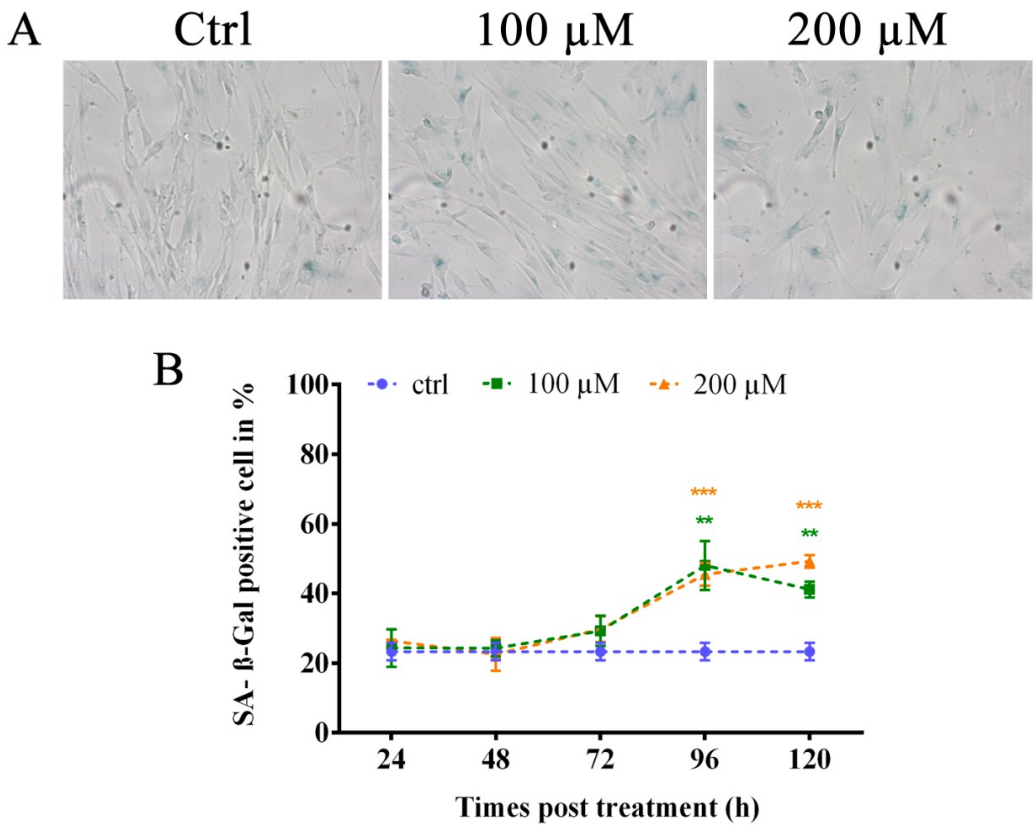


Figure 5. Analysis of SA-β-galactosidase-positive cells. (**A**) Representative images of positive SA-β-Gal-positive cells. (**B**) The graph represents data obtained from the analysis of the percentage of SA-β-galactosidase-positive cells. Control values are shown as the mean of the controls analyzed at different times. We observed a significant increase in the percentage of SA-β-Gal-positive cells at 96 h after treatment at both doses of hydrogen peroxide that persisted at 120 h after treatment. The error bars denote the standard error. ** $p < 0.01$; *** $p < 0.001$ by Student's *t*-test.

Coluzzi et al., 2019

Indicatori di senescenza

H_2O_2-induced senescence

Biochemical characteristics of cellular senescence:
-elevated protein levels of p53, $p16^{INK4a}$, promyelocytic leukemia gene product PML
-senescence-associated h-galactosidase (SA-h-gal) activity assayed at an acidic pH
-No BrdU incorporation

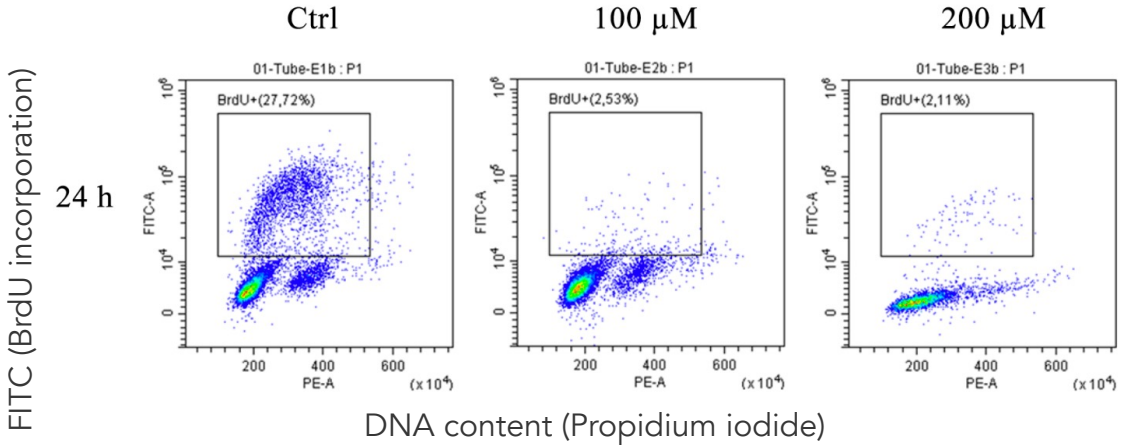


Figure 6. Cytometric analysis of BrdU incorporation. The incorporation of BrdU was used to detect the cell proliferation status by flow cytometry. In this figure, biparametric dot plots of control or H_2O_2-treated MRC-5 cells show that this treatment, at 24 h (**A**), completely arrested cell proliferation. Which was slightly recovered at 48 h (**B**). The magnitude of this cell cycle arrest effect is dose related. The box gate on dot plots represents BrdU-positive cells.

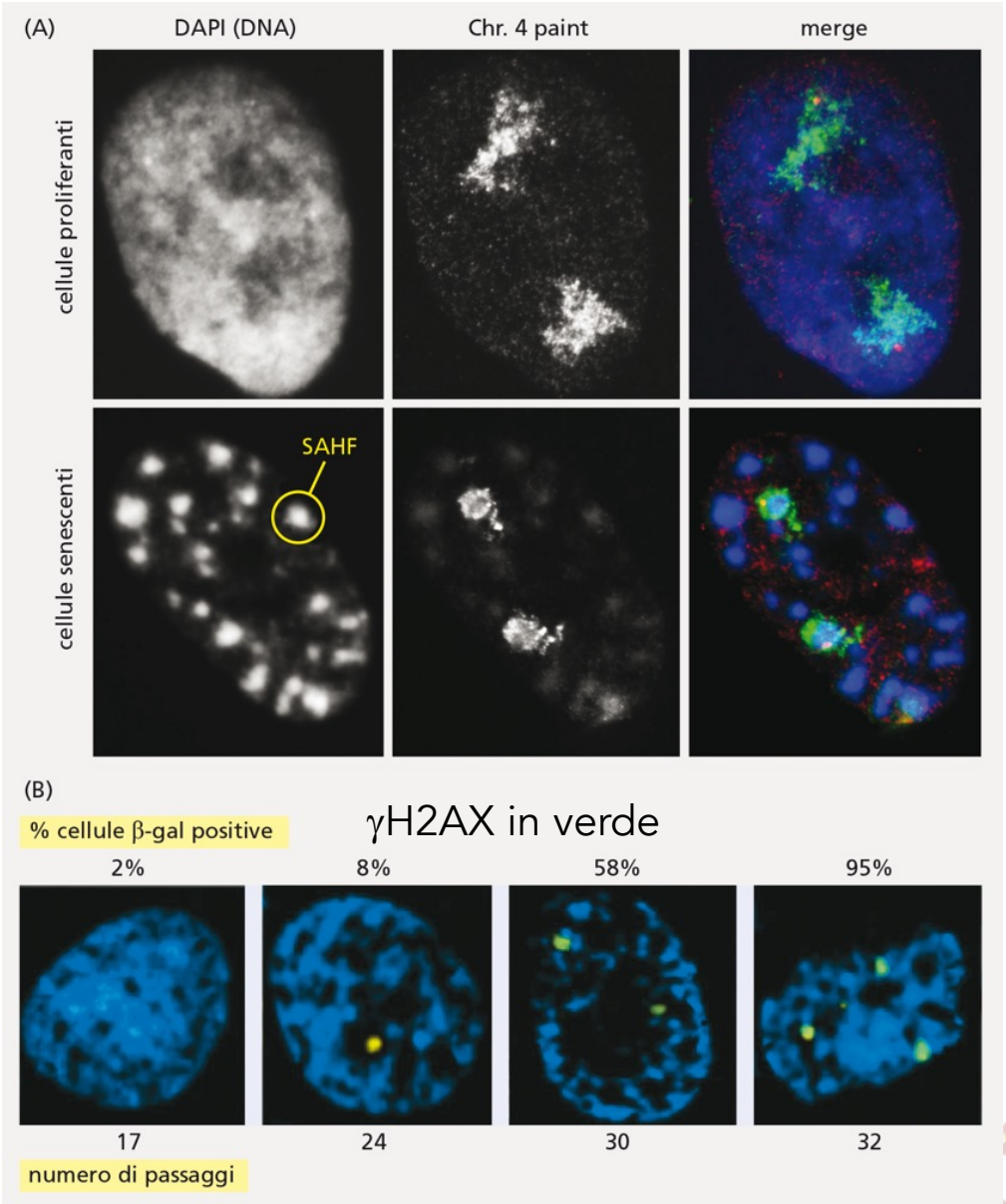
Coluzzi et al., 2019

Marcatori molecolari di senescenza

β-galattosidasi non è specifico perché può essere espressa anche da cellule senescenti

Foci di eterocromatina (SAHF) dipendenti da pRb

Es. anticorpi contro H2K9me o foci di danno (γH2AX)



Weinberg, la biologia del cancro

Marcatori molecolari di senescenza

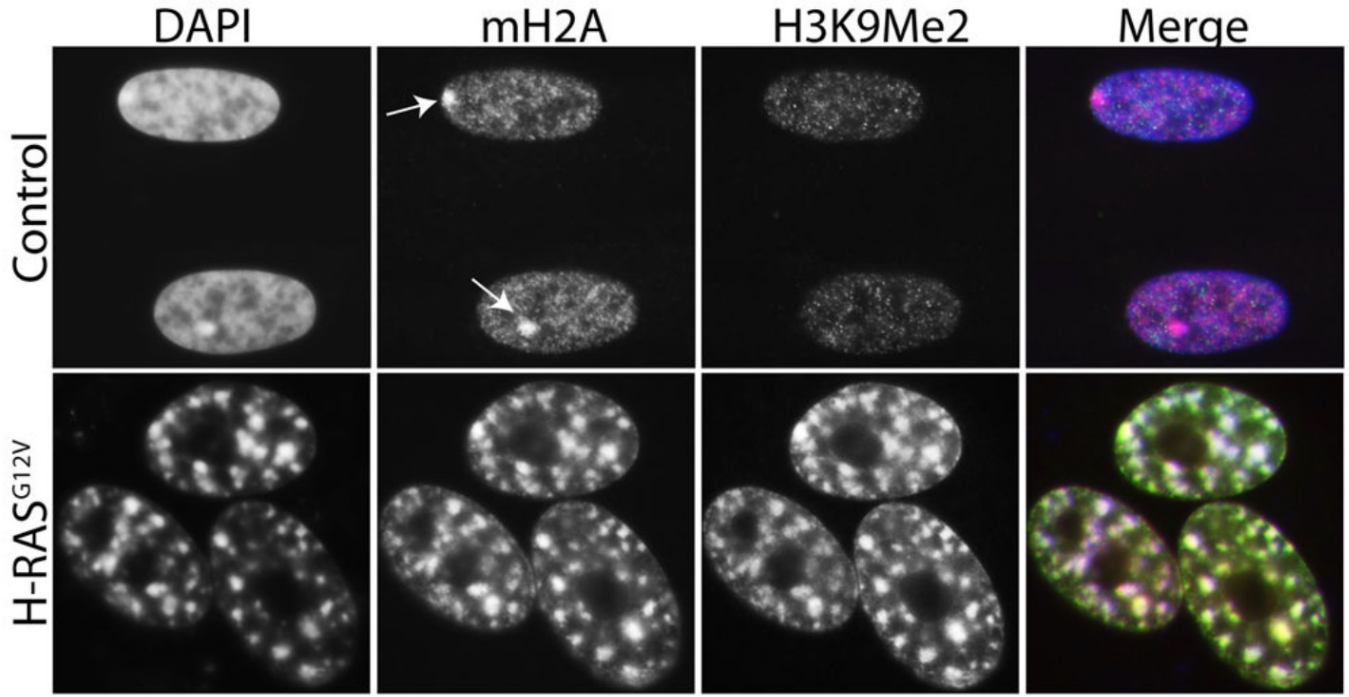
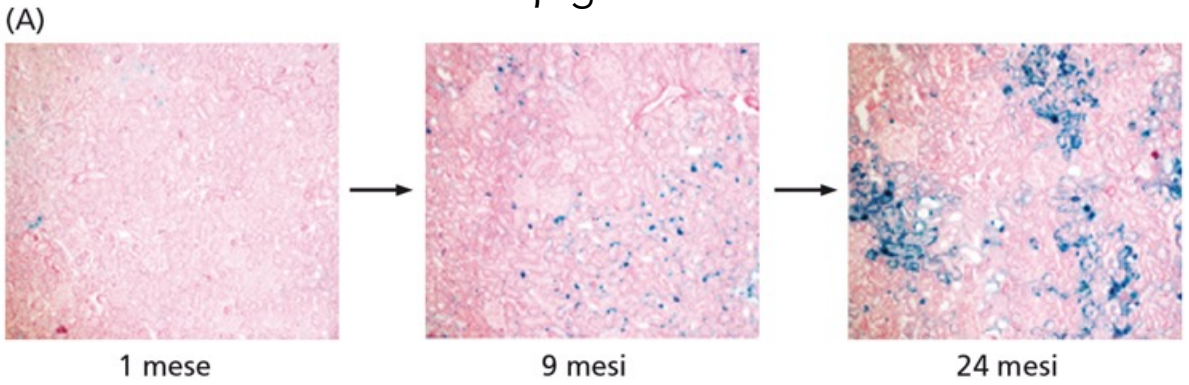


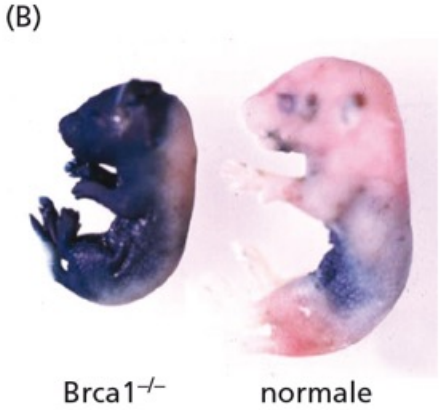
Fig. 1.
IMR90 cells were infected with a puromycin-resistant retrovirus encoding oncogenic H-RAS^{G12V} or vector control. Drug-selected cells were stained with antibodies to H3K9Me2 and histone H2A variant macroH2A. DAPI counterstaining was used to visualize SAHF. Note the robust punctate DAPI foci in the H-RAS^{G12V} infected cells, which co-localize with both macroH2A and H3K9Me2. Arrows point to inactivated X chromosome in control IMR90 female fibroblast cells. Chicken anti-macroH2A1.2 primary antibody was obtained from Dr. John R. Pehrson (University of Pennsylvania) (28). Rabbit anti-H3K9Me3 is from AbCam (ab8898) and was used at 1:500. Secondary antibodies were FITC-labeled goat anti-chicken (1:2500) and Cy3-labeled goat anti-rabbit (1:5000), which were both obtained from Jackson Immunolabs.

Aird and Zhang, 2013

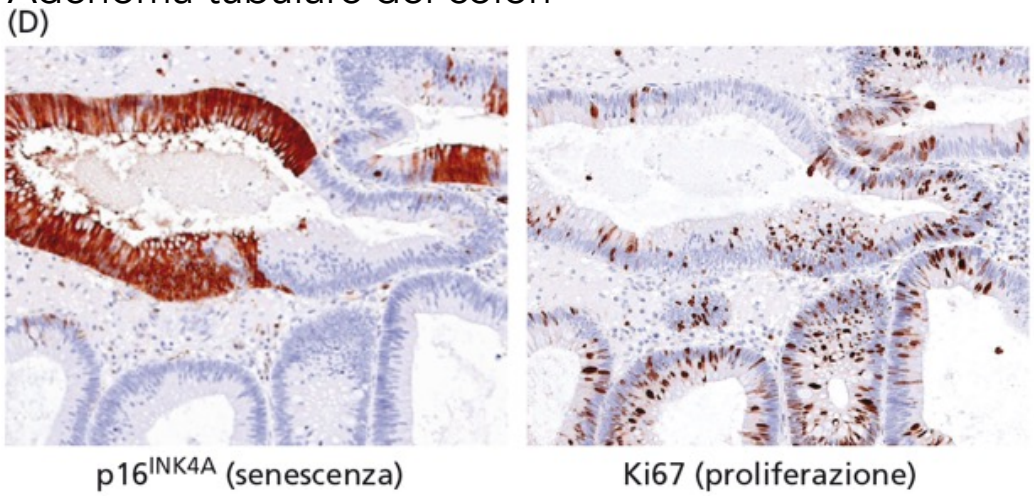
Monitorare la senescenza in vivo

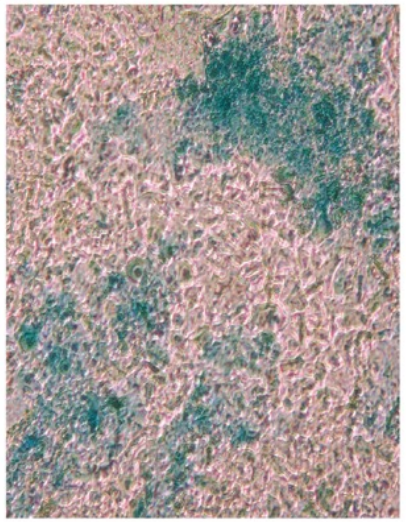
Rilevamento attività β-galattosidasi. Rene normale





Rilevamento proteine che arrestano la proliferazione. Adenoma tubulare del colon

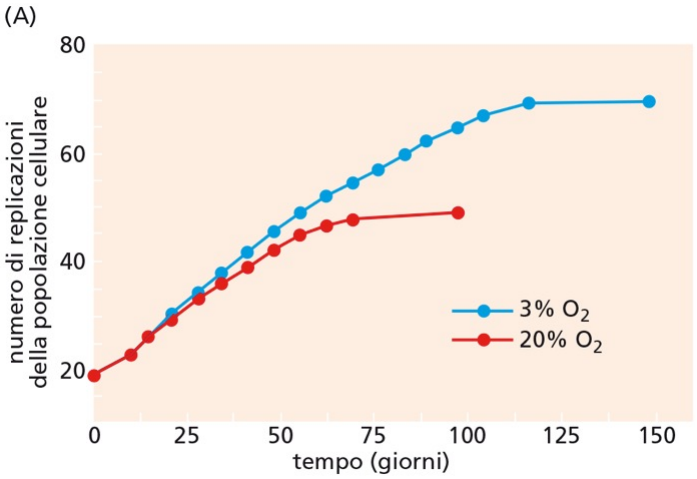


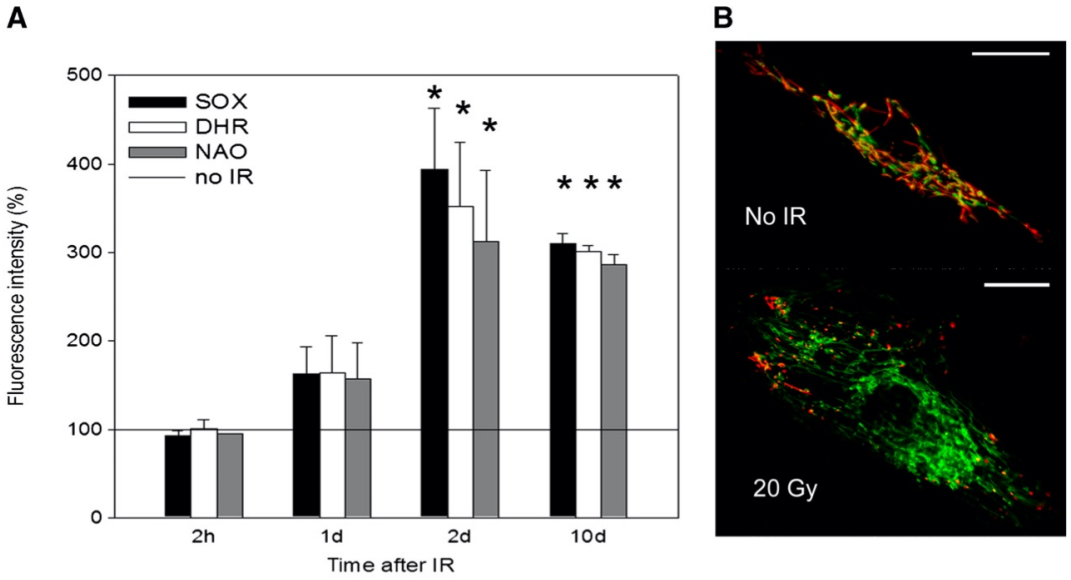


Tumore polmonare + cisplatino e taxolo

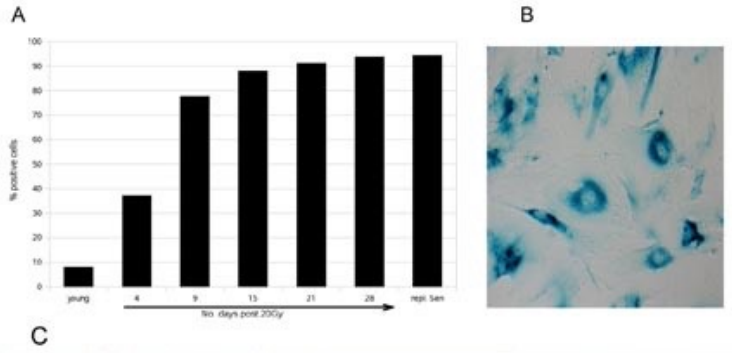
Weinberg, la biologia del cancro

Segnali che inducono senescenza



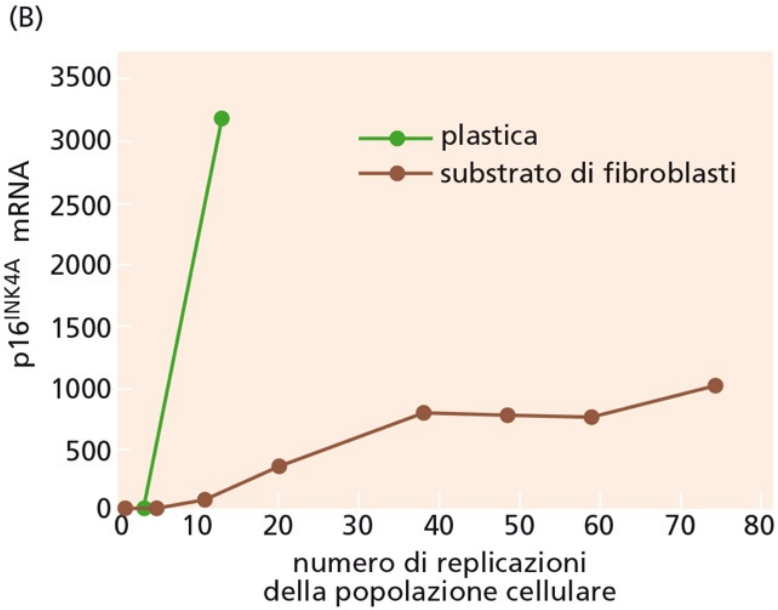


Accumulo Specie Reattive dell'ossigeno
In senescenza



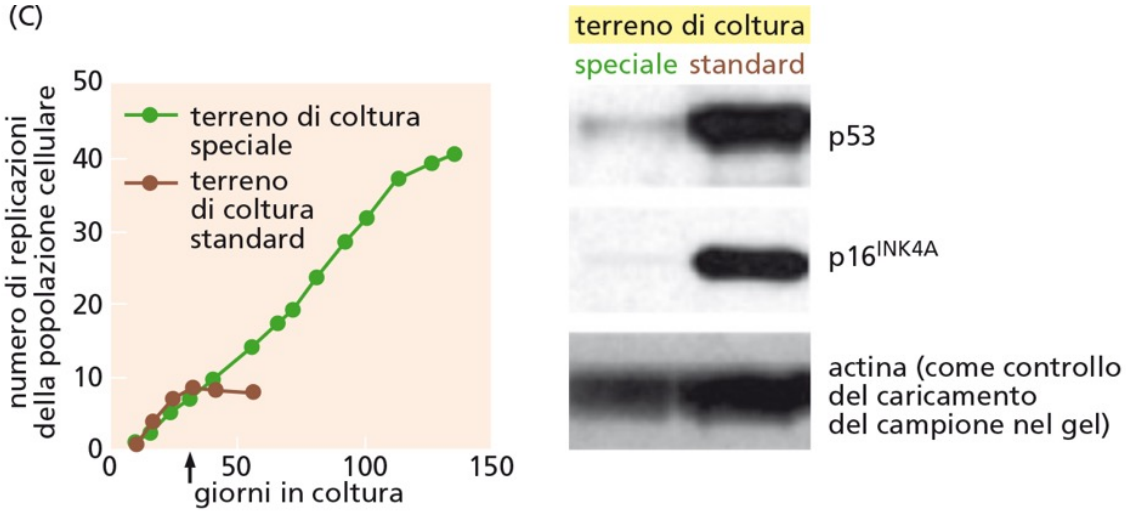
Weinberg, la biologia del cancro; Passos et al., 2010

Segnali che inducono senescenza



Senescenza di cellule epiteliali in coltura

Senescenza non dipende solo dal tempo ma dalle condizioni di stress



Weinberg, la biologia del cancro